Leaf physiology, production, water use, and nitrogen dynamics of the grassland invader *Acacia smallii* at elevated CO₂ concentrations

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Summary   Invasion by woody legumes can alter hydrology, nutrient accumulation and cycling, and carbon sequestration on grasslands. The rate and magnitude of these changes are likely to be sensitive to the effects of atmospheric CO_2 enrichment on growth and water and nitrogen dynamics of leguminous shrubs. To assess potential effects of increased atmospheric CO_2 concentrations on plant growth and acquisition and utilization of water and nitrogen, seedlings of *Acacia smallii* Isely (huisache) were grown for 13 months at CO_2 concentrations of 385 (ambient), 690, and 980 μmol mol^{-1}. Seedlings grown at elevated CO_2 concentrations exhibited parallel declines in leaf N concentration and photosynthetic capacity; however, at the highest CO_2 concentration, biomass production increased more than 2.5-fold as a result of increased leaf photosynthetic rates, leaf area, and N_2 fixation. Measurements of leaf gas exchange and aboveground biomass production and soil water balance indicated that water use efficiency increased in proportion to the increase in atmospheric CO_2 concentration. The effects on transpiration of an accompanying decline in leaf conductance were offset by an increase in leaf area, and total water loss was similar across CO_2 treatments. Plants grown at elevated CO_2 fixed three to four times as much N as plants grown at ambient CO_2 concentration. The increase in N_2 fixation resulted from an increase in fixation per unit of nodule mass in the 690 μmol mol^{-1} CO_2 treatment and from a large increase in the number and mass of nodules in plants in the 980 μmol mol^{-1} CO_2 treatment. Increased symbiotic N_2 fixation by woody invaders in response to CO_2 enrichment may result in increased N deposition in litterfall, and thus increased productivity on many grasslands.

Keywords: huisache, nitrogen fixation, photosynthesis, stomatal conductance, water use efficiency.

Introduction

The abundance and density of C₃ shrubs on predominantly C₄ grasslands in many parts of the world (Africa, Australia, North America, South America) have increased dramatically during the last 125 years (reviewed by Mayeux et al. 1991, Archer 1994). Woody invasion changes the composition and structure of grassland communities and may also alter ecosystem properties. Because woody species differ from grasses in mature size, growth form, and physiology, they may alter trophic-level interactions and disturbance regimes on grasslands. Shrubs, for example, may reduce fire frequencies and intensities and, in some systems, decrease livestock production by competitively displacing shorter grasses and other herbaceous species (Hobbs and Mooney 1986, Heitschmidt and Dowhower 1991). In other systems, often with lower densities of woody species, productivity of herbaceous vegetation is higher under woody canopies than in open grassland (Belsky et al. 1989, Weltzin and Coughenour 1990). Because shrubs and grasses, especially C₄ grasses, sometimes differ in timing and patterns of resource acquisition or in resource use efficiency, invasion by shrubs can also alter hydrology (Joffre and Rambal 1993), potential productivity (Belsky et al. 1989), nutrient accumulation and cycling (Stock et al. 1995), and rates and patterns of carbon sequestration on grasslands (McPherson et al. 1993, H.B. Johnson, unpublished data). The spatial distribution of nutrients and water in these ecosystems may also be modified (Belsky et al. 1989, Schlesinger et al. 1990).

The rate and magnitude of changes following woody species ingress depend on the specific morphologies, growth rates, and physiologies of the invasive shrubs. Changes on grasslands may be particularly pronounced following invasion by deeply rooting and rapidly growing woody legumes, because such species may both deplete surficial water that would otherwise support growth of grasses, and access deep soil water not readily available to grasses (Dugas and Mayeux 1991). By adding fixed N_2 to the system (Johnson and Mayeux 1990, Stock et al. 1995, Maron and Connors 1996), woody legumes could also influence the productivity and species composition of grasslands.

The increase in atmospheric CO_2 concentration projected for the next century may modify the impact of woody invaders on grasslands by altering the growth rates of shrubs and their acquisition and utilization of essential plant resources such as water and nitrogen (N) (e.g., Polley et al. 1994). Rising atmospheric CO_2 concentration may reduce transpiration and increase the water use efficiency of woody and other plants by inducing stomatal closure (Morison 1987, Field et al. 1995). However, any effect of CO_2 concentration on transpiration will also depend on the responses of plant growth and leaf area to CO_2, both of which may be limited by soil N availability. Soil N availability may be less limiting to legumes than to other

plants if, as is often found, increased CO_2 concentration stimulates N_2 fixation by the legume–*Rhizobium* symbiosis (Thomas et al. 1991, Vogel and Curtis 1995).

We investigated effects of elevated CO_2 concentration on growth, water use and water use efficiency, and nitrogen economy of the deeply rooting woody plant *Acacia smallii* Isely ((huisache); formerly considered a part of *A. farnesiana*). We also explored possible feedbacks of exposure to elevated CO_2 on stomatal conductance and leaf photosynthesis that may modify or limit the long-term impact of this species on grasslands.

Acacia smallii is an aggressive grassland invader that achieves maximum growth in full sunlight (Bush and Van Auken 1986, Lohstroh and Van Auken 1987), but may establish in ungrazed grassland (Meyer and Bovey 1982). The shrub occurs in southern Texas in the Rio Grande plains and coastal plains along the Gulf of Mexico, and ranges southward to Mexico and eastward along the gulf coast of Louisiana (Isely 1973). Annual precipitation in these areas ranges from about 450 mm in the west to 1200 mm in the eastern coastal regions (Arbingast et al. 1979).

Materials and methods

Species and culture

During August 1992, seeds of *A. smallii* were planted in fine sandy loam soil (Udic Paleustalfs; Huckabee et al. 1977) in wheeled, 380-liter containers (0.9 m deep and 0.65 m on each side). Three *A. smallii* plants were established in each container. Three individuals each of the C_3 grass *Stipa leucotricha* Trin. & Rupr. (Texas winter grass) and the C_4 grass *Schizachyrium scoparium* (Michx.) Nash. (little bluestem) were also established from tillers in each container to provide a competitive environment for *A. smallii* growth. In addition, six plants of each species were grown singly in 30-liter pots, and used to study root biomass. Organic matter of the fine sandy loam soil was enriched in ^{15}N by mixing finely ground grass plants labeled with ^{15}N (7.54% of total soil N) with the soil (Polley et al. 1992). Total soil N at planting was 0.03%. No fertilizer was added.

Two 30-liter pots of each species and three 380-liter containers of the species mix were maintained for 13 months at average CO_2 concentrations of 385 (ambient), 690, or 980 $\mu mol\ mol^{-1}$ in air-conditioned greenhouse bays. To minimize any effect of bay position on plant performance, plants and CO_2 treatments were moved monthly among bays until June 1993. Thereafter, plants were too large to move through adjoining doorways.

Water was added weekly from October 1992 through August 1993 and every 4 days from August 1993 until harvest in October 1993 to bring soil to field capacity (approximately 20% volumetric soil water content). Soil water content was measured through the sides of the 380-liter containers with a surface moisture gauge (Model 3218, Troxler Electronics, Research Triangle Park, NC). An empirically determined relationship between the volume of water lost per container and the decline in gauge reading from that measured when soil was at field capacity was used to determine water additions. To determine required water additions for 30-liter pots, the pots were weighed with a load beam (Model KIS-3, BLH Electronics, Canton, MA).

Carbon dioxide treatments and environmental conditions

Carbon dioxide gas was injected into the greenhouse bays as necessary to maintain the desired concentrations. The CO_2 concentration and dewpoint temperature of air in each bay were measured at 4-min intervals with an infrared gas analyzer (Model 6262, Li-Cor, Inc., Lincoln, NE). The CO_2 readings were corrected for atmospheric pressure measured with a pressure indicator (Model DPI 260, Druck Inc., New Fairfield, CT). Air temperature in each bay was changed seasonally to approximate outdoor temperature by manually adjusting thermostatic controls, and was measured with 25-μm diameter thermocouples. Photosynthetic photon flux density (PPFD) was measured on the greenhouse roof with a silicone photodiode (Model LI190SB, Li-Cor, Inc.), and above plants in each bay with silicone detectors along a 1-m long sensing surface (Model LI191SA, Li-Cor, Inc.).

The CO_2 concentration of air averaged 385, 690, and 980 $\mu mol\ mol^{-1}$ for the three CO_2 treatments. Standard deviations of CO_2 concentrations were calculated daily. The average of these values ranged from 11.4 $\mu mol\ mol^{-1}$ at the mid-level CO_2 concentration to 23.1 $\mu mol\ mol^{-1}$ at the ambient CO_2 concentration. Mean daytime temperatures declined from 30.0 °C in August 1992 to 16.8 °C in January 1993, and then increased linearly with time to 27.5 °C at harvest in October 1993. The vapor pressure deficit of air followed a similar temporal trend. Vapor pressure deficit during daylight declined from a mean of 2.0 kPa in August 1992 to 0.6 kPa in January 1993, and then increased linearly with time to 1.4 kPa in October 1993. The daily integral of PPFD inside the bays averaged 56% of that measured above the greenhouse, although instantaneous PPFD inside the greenhouse bays approached 90% of that outdoors at midday.

Leaf gas exchange

Net photosynthesis and stomatal conductance to water vapor were measured on *A. smallii* seedlings grown in the 380-liter containers. Measurements were taken on fully expanded and sunlit leaves on clear days during August and September 1993 with a portable gas exchange system (Model MPH1000, Campbell Scientific, Logan, UT), coupled to a Li-Cor Model 6262 infrared analyzer. One set of leaves was measured near the CO_2 concentration that prevailed during growth (350, 700 or 1000 $\mu mol\ mol^{-1}$). The steady-state response of gas exchange to varying CO_2 concentrations was measured on a second set of leaves from each treatment. Each leaf was exposed in sequence to CO_2 concentrations of 350, 700, and 1000 $\mu mol\ mol^{-1}$. Photosynthesis and stomatal conductance typically stabilized within 15 min following a change in CO_2 concentration. Gas exchange was measured at a leaf temperature of 28 °C and a leaf-to-air vapor pressure difference of 1.7 kPa. Incident PPFD averaged 1355 $\mu mol\ m^{-2}\ s^{-1}$ across measurements.

Leaves for which gas exchange was measured were collected, and leaf area determined with a photoelectric area meter. Individual leaves were dried, weighed, and analyzed for N content following Dumas combustion (Morris et al. 1968). Instantaneous gas exchange parameters were calculated based on one-sided leaf area (von Caemmerer and Farquhar 1981).

Harvest and analyses

Aboveground tissues of *A. smallii* were harvested between October 1 and 4, 1993, dried to constant mass at 60 °C, and weighed. Leaf area per plant was calculated as the product of leaf mass and the specific leaf area of a subsample of leaves from each plant. There were two periods before harvest during which some plants lost some of their leaves. Abscised leaves were collected from the concrete floor of the greenhouse, dried, and weighed.

Four soil cores, each 4.5 cm in diameter, were taken from each 380-liter container at harvest in October 1993. Single cores were also taken from two 30-liter pots of each species grown at each CO_2 concentration. Roots were separated from the soil by hand, and nodules were separated from roots and counted. Roots were washed, shaken for 1 h in 0.1 M HCl to remove adhering soil carbonates, dried at 60 °C, and weighed.

The contribution of *A. smallii* roots to total root biomass in the 380-liter containers was calculated from root ^{13}C and $\%^{15}N$ signatures (Polley et al. 1992). Isotopic signatures were measured by isotope ratio mass spectrometry (Isotope Services, Inc., Las Alamos, NM). The mass of ^{13}C or ^{15}N in roots of a species mixture is the sum of the products of the ratios of ^{13}C to total C or ^{15}N to total N (derived from ^{13}C or $\%^{15}N$ analyses) and root C or N masses of the component species (biomass × % elemental composition). Identifiable roots of *A. smallii* in cored soil from each container were analyzed. To minimize disturbance to planted containers, the carbon and nitrogen concentrations and isotopic signatures of roots of *S. leucotricha* and *S. scoparium* in the 380-liter containers were estimated from those of plants grown in the 30-liter containers at each CO_2 concentration. The ratio of the ^{13}C, $\%^{15}N$, [N], or [C] value of roots to leaves of each species in the 30-liter pots was multiplied by the mean value of leaves for each species in the mixtures. Root biomass of the C_4 species *S. scoparium* was algebraically distinguished from that of the two C_3 species by its distinctive ^{13}C signature. Because atmospheric N_2 fixed by *A. smallii* in symbiosis with *Rhizobium* was depleted in ^{15}N relative to that in soil, roots of the legume could be distinguished from those of *Stipa* by their respective $\%^{15}N$ values.

Kjeldahl N was measured on leaves and aboveground stems of *A. smallii* harvested in October 1993 and on leaves that abscised before harvest. Because the samples of roots were usually too small for Kjeldahl analyses, the N concentrations of these samples and the C concentrations of all tissues were analyzed following Dumas combustion (Morris et al. 1968). There was a strong correlation between the results of the two methods of N analysis ($r^2 = 0.998$, $n = 6$). This correlation was used to standardize N concentrations determined following combustion to those derived from Kjeldahl analysis. Nitrogen masses of tissues of the three *A. smallii* plants per 380-liter

container (biomass × [N]) were summed to determine nitrogen accretion per container.

The fraction of N in *A. smallii* that was derived from N_2 fixation was estimated from leaf $\%^{15}N$ values (Shearer and Kohl 1986). The isotopic signature of *A. smallii* that depended entirely on atmospheric N_2 as an N source was assumed to be equal to -1.3 $\delta^{15}N$ or 0.3658% ^{15}N (Shearer et al. 1983). Because the C_4 grass *S. scoparium* grew during the period of greatest growth by *A. smallii*, we used the average $^{15}N/^{14}N$ signature of upper-canopy leaf blades from the three *S. scoparium* plants per 380-liter container as an estimate of the isotopic signature of soil N available to neighboring shrubs.

Nitrogen accretion by each *A. smallii* plant was multiplied by the proportion of N derived from fixation to calculate the mass of N fixed per plant. Nitrogen fixed by each of the three *A. smallii* plants per 380-liter container was summed and subtracted from total N accretion per container to estimate the mass of shrub N derived from soil uptake.

Apparent water use efficiency of *A. smallii* was calculated as the ratio of total biomass production (including abscised leaves) of woody plants per soil container to evapotranspiration. Because grasses contributed 15% or less to total leaf area in containers throughout the study, we assumed that grasses contributed little to total transpiration.

Analysis of variance with repeated measures (Potvin et al. 1990) was used to discern effects of growth and measurement CO_2 concentration on leaf gas exchange. Remaining data were evaluated by univariate analysis of variance. Student-Newman-Keuls' multiple range test was used to determine significant differences among three means. Data were logarithmically transformed before analysis when assumptions of ANOVA were violated by nontransformed data. Standard errors for transformed data are omitted in presentation of results using the original scale of measurement.

Results

Leaf gas exchange and water use efficiency

Elevated CO_2 concentrations caused a decline in stomatal conductance of *A. smallii* grown in species mixtures (Table 1), whereas leaf net photosynthesis was increased by CO_2 enrichment. Photosynthetic water use efficiency (net photosynthesis/transpiration; PWUE), or transpiration efficiency, of *A. smallii* increased in proportion with increasing CO_2 concentration, with the result that c_i/c_a remained almost constant among leaves that were grown and measured at different CO_2 concentrations. Nitrogen per unit area was similar for leaves grown at the two highest CO_2 concentrations, but was significantly greater in leaves that were grown and measured near the ambient concentration.

A nearly constant c_i/c_a of about 0.75 (data not shown) was also maintained when individual leaves were measured at CO_2 concentrations from 350 to 1000 $\mu mol\ mol^{-1}$, regardless of the growth CO_2 concentration. At all measurement CO_2 concentrations, net photosynthesis was highest in leaves grown near ambient CO_2 concentration ($P = 0.02$), but photosynthesis of ambient-grown leaves changed little between measurement

Table 1. Net photosynthetic rates, stomatal conductance, photosynthetic water use efficiency (PWUE), nitrogen concentrations, and ratios of intercellular to ambient CO_2 concentration (c_i/c_a) for leaves of *A. smallii* that were grown and measured near 385 μmol mol^{-1} ($n = 26$), 690 μmol mol^{-1} ($n = 25$) and 980 μmol mol^{-1} CO_2 ($n = 18$). Gas exchange was measured following one year at the different CO_2 treatments at a leaf-to-air vapor pressure difference of 1.7 kPa and a mean incident PPFD of 1344 μmol m^{-2} s^{-1}. Means within a row do not differ significantly ($P > 0.05$) if followed by the same letter.

Parameter	Growth CO_2 concentration (μmol mol^{-1})		
	385	690	980
Net photosynthesis (μmol m^{-2} s^{-1})	13.4 a	16.3 a	20.8 b
Stomatal conductance (mmol m^{-2} s^{-1})	275.4 a	189.6 b	152.7 b
PWUE (mmol mol^{-1})	3.13 a	5.18 b	8.55 c
c_i/c_a	0.73 a	0.77 a	0.74 a
Leaf N (g m^{-2})	2.8 a	1.4 b	1.5 b

Figure 1. Net photosynthesis as a function of short-term measurement CO_2 concentration for leaves of *A. smallii* that were grown at 385 μmol mol^{-1} ($n = 11$), 690 μmol mol^{-1} ($n = 10$), and 980 μmol mol^{-1} CO_2 ($n = 10$). Photosynthesis of each leaf was measured at all CO_2 concentrations. Incident PPFD averaged 1366 μmol m^{-2} s^{-1}. Bars indicate 1 standard error of the mean at each measurement concentration.

CO_2 concentrations of 700 and 1000 μmol mol^{-1} (Figure 1). Net photosynthesis of plants that were grown in elevated CO_2 concentrations increased linearly with short-term increases in measurement CO_2 concentration. Because of these differences in photosynthetic response to CO_2, the differences in net photosynthesis between leaves grown at ambient and elevated CO_2 concentrations decreased as the measurement CO_2 concentration increased. Net photosynthesis measured at 350 μmol mol^{-1} was 75% greater in leaves grown at 385 μmol mol^{-1} CO_2 than in leaves grown at higher concentrations, but photosynthetic rates of leaves grown near ambient CO_2 concentration were only 48 and 17% greater than photosynthetic rates of leaves grown at elevated CO_2, when measured at 700 and 1000 μmol mol^{-1} CO_2, respectively.

Leaf N per unit area was significantly higher for plants grown at 385 μmol mol^{-1} CO_2 than at the two elevated CO_2 concentrations (Figure 2). There was a significant linear relationship between photosynthesis at each measurement CO_2 concentration and leaf N per unit area when data from plants grown at the three CO_2 concentrations were pooled. However, the variance in photosynthesis explained by leaf N decreased from 0.79 to 0.32 as CO_2 concentration increased from 350 to 1000 μmol mol^{-1}, demonstrating that N alone accounted for only a portion of the difference in net photosynthesis among leaves from the different CO_2 treatments.

Growth at elevated CO_2 altered the stomatal response to short-term changes in measurement CO_2 (Figure 3). Stomatal conductance was higher in leaves grown at ambient CO_2 than at elevated CO_2 concentrations, regardless of measurement concentration. The value of leaf c_i/c_a was similar across measurement and growth conditions, indicating that changes in stomatal conductance were highly correlated with changes in net photosynthesis.
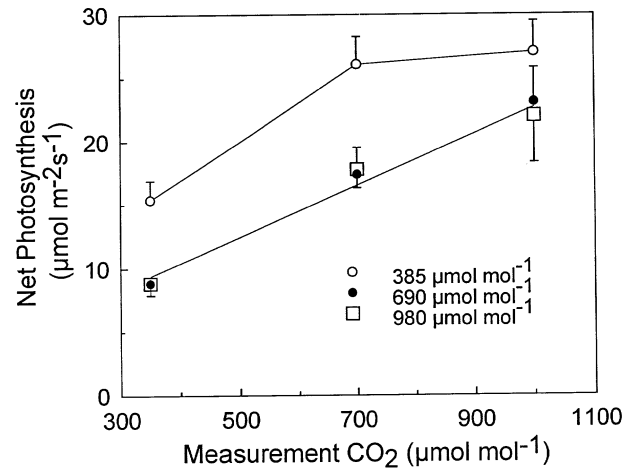

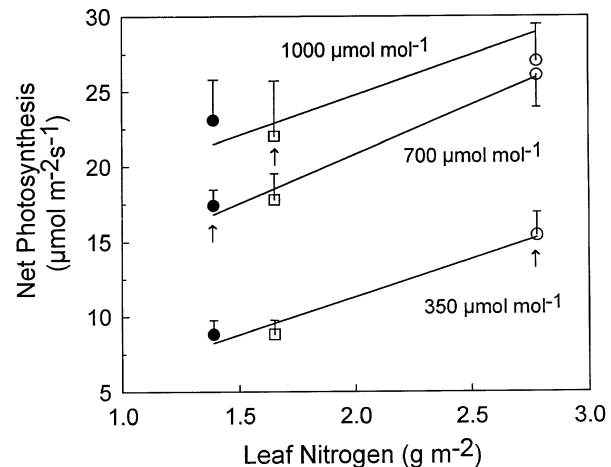
Figure 2. Mean net photosynthetic rates measured at 350, 700, and 1000 μmol mol^{-1} as a function of the mean nitrogen concentration of leaves from *A. smallii* grown at 385 μmol mol^{-1} (○, $n = 11$), 690 μmol mol^{-1} (●, $n = 10$), and 980 μmol mol^{-1} CO_2 (□, $n = 10$). Bars indicate 1 standard error of the mean at each measurement concentration. Lines were derived from linear regressions that included net photosynthesis and leaf nitrogen of individual leaves at each CO_2 concentration. Arrows denote mean photosynthetic rates near the CO_2 concentration that prevailed during growth.

Plant biomass and water use efficiency

Total biomass production increased in proportion to increases in growth CO_2 concentration: 87% biomass increase from 385 to 690 μmol mol^{-1} CO_2, and a further 34% biomass increase from 690 to 980 μmol mol^{-1} CO_2 (Table 2). Most of the increase in biomass occurred aboveground. Stem biomass production increased 77% from 385 to 690 μmol mol^{-1} CO_2, and 30% from 690 to 980 μmol mol^{-1} CO_2, but the largest relative
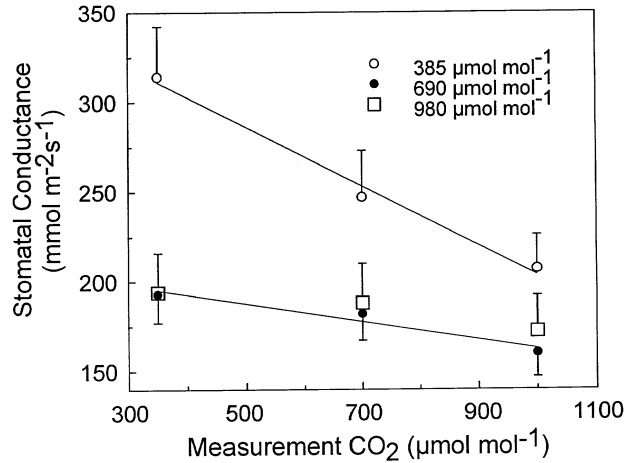
Figure 3. Stomatal conductance as a function of short-term measurement CO_2 concentration for leaves grown at 385 μmol mol^{-1} ($n = 11$), 690 μmol mol^{-1} ($n = 10$), and 980 μmol mol^{-1} CO_2 ($n = 10$). Conductance of individual leaves was measured at each concentration. Bars indicate 1 standard error of the mean.

Table 2. Biomass production and apparent water use efficiency (WUE) of *A. smallii* after 13 months of growth at each of three CO_2 concentrations. Values for each CO_2 treatment are means for three 380-liter soil containers, each with three plants. Means within a row do not differ significantly ($P > 0.05$) if followed by the same letter.

Parameter	Growth CO_2 concentration (μmol mol^{-1})		
	385	690	980
Biomass production (g)			
Stems	1616.0 a	2868.2 b	3741.7 c
Leaves	407.0 a	766.1 a	1294.4 b
Total aboveground	2023.0 a	3634.3 b	5036.1 c
Roots	266.3 a	649.2 b	724.1 b
Total plant	2289.3 a	4283.5 b	5760.2 c
WUE (g l^{-1})	1.77 a	3.63 b	4.78 c

increase in aboveground production occurred in leaves. This trend was most marked in shrubs grown at the highest CO_2 concentration, which lost a mean 42% of total leaf production during two episodes of leaf drop. Less than 20% of total leaf production was lost from plants that were grown at the lower CO_2 concentrations.

Total evapotranspiration from containers with *A. smallii* was remarkably constant and differed by only 10% among CO_2 treatments (data not shown). As a result, apparent water use efficiency (the ratio of biomass production to evapotranspiration) increased in proportion with increased CO_2 concentration: 105% increase in WUE from 385 to 690 μmol mol^{-1} CO_2, and 32% increase in WUE from 690 to 980 μmol mol^{-1} CO_2 (Table 2).

Nitrogen dynamics

Total N accretion by *A. smallii* increased with each increase in CO_2 concentration: 55% from 385 to 690 μmol mol^{-1} CO_2, and another 29% from 690 to 980 μmol mol^{-1} CO_2 (Figure 4). Nitrogen accretion in individual tissues changed less consistently. For example, nitrogen in aboveground stems increased with each increase in CO_2 concentration, but the N content of leaves present at harvest did not differ with CO_2 treatment. As a result, leaf N was a smaller fraction of both aboveground and total N present at harvest in plants grown at elevated CO_2 concentrations than at ambient concentration (data not shown). However, because plants grown at the highest CO_2 concentration lost 27% of leaf N to leaf abscission during the year, total N accretion in leaves was significantly higher in plants grown at 980 μmol mol^{-1} CO_2 than at ambient CO_2 concentration.

At elevated CO_2 concentrations, the increase in N accretion did not match the increase in biomass production because there was a decline in the N concentration of leaves and aboveground stems. Specific area of leaves present at harvest was not altered by CO_2 concentration (mean = 0.016 m^2 g^{-1}), so N per
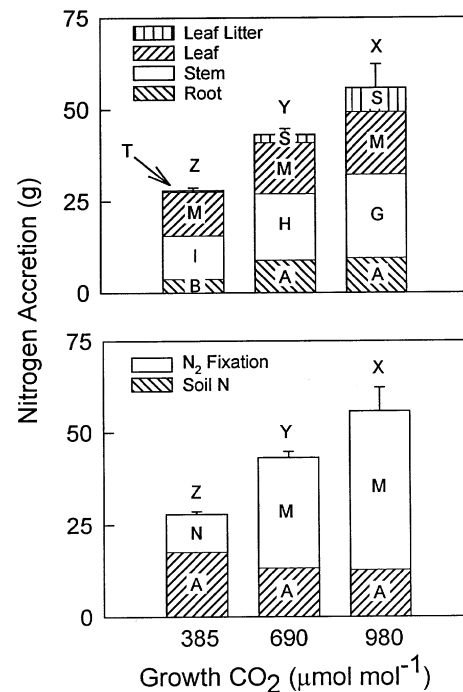

Figure 4. Total N accretion by *A. smallii* grown from seed at 385, 690 and 980 μmol mol^{-1} CO_2 and its division among components. Values represent N accretion of the three plants per 380-liter soil container ($n = 3$ containers per CO_2 treatment). Total N accretion by whole plants and plant components (upper panel) and total N accretion by N source (lower panel). Column or component values differing significantly ($P > 0.05$) across CO_2 treatments are labeled with different letters. Error bars indicate 1 standard error of total N accretion.

unit area also declined at elevated CO_2, which is consistent with the gas exchange measurements.

In the elevated CO_2 treatment, the fraction of N derived from N₂ fixation, calculated from leaf % ^{15}N values (Table 3), was about twice that of plants grown near the ambient CO_2 concentration. Because shrubs grown at elevated CO_2 also contained more total N, the mass of plant N derived from fixation at

Table 3. Values of %^{15}N of upper canopy leaves of *S. scoparium* and *A. smallii* grown for 13 months in ^{15}N-enriched soil at each of three CO_2 concentrations. Values are means for three plants of each species from each of three 380-liter soil containers per CO_2 treatment.

CO_2	Leaf %^{15}N	
(μmol mol^{-1})	*Schizachyrium scoparium*	*Acacia smallii*
385	0.5635	0.4933
690	0.5694	0.4406
980	0.5504	0.4113

elevated CO_2 was 3 to 4 times that fixed at ambient CO_2 concentration (Figure 4). This increase in N_2 fixation resulted from an increase in fixation per nodule at 690 μmol mol^{-1} CO_2, and from an increase in the number and mass of nodules at 980 μmol mol^{-1} CO_2 (Table 4). Despite the large increase in root biomass at elevated CO_2 concentration, the CO_2 treatments did not significantly affect the mass of N that *A. smallii* obtained from soil.

Discussion

Increasing atmospheric CO_2 concentration to 980 μmol mol^{-1} increased biomass production of the woody legume *Acacia smallii* more then 2.5-fold during the initial year of growth by increasing leaf photosynthetic rates, leaf area, and N_2 fixation. The increase in biomass at elevated CO_2 required no more water than was consumed by the shrubs grown near ambient CO_2 concentration. As a result, apparent water use efficiency and biomass production increased by similar relative amounts.

The absolute increase in biomass production at elevated CO_2 would have been smaller if water had been limiting. It is not apparent, however, what effect water limitation would have had on the relative increase in biomass at elevated CO_2 concentrations. Guehl et al. (1994) found that the relative increase in biomass of two tree species in response to elevated CO_2 was less in drought-treated plants than in well-watered plants. In many studies, however, the relative enhancement in biomass at elevated CO_2 under water-limiting conditions is as great as, or greater than, the enhancement under well-watered conditions (Conroy et al. 1988, Morison 1993, Townend 1995).

Despite the marked growth response of *A. smallii* to elevated CO_2, there was evidence that the response was limited by low soil N availability combined with an imperfect coupling between C uptake and N_2 fixation. Photosynthetic capacity of *A. smallii* adjusted downward following 13 months at elevated CO_2, as is commonly observed when non-N_2 fixing plants are grown in pots (Arp 1991). Photosynthetic rates were correlated with leaf N when measured at a common CO_2 concentration, suggesting that the decline in photosynthetic capacity at elevated CO_2 was partially explained by lower leaf N per unit area, and did not simply reflect reallocation of N among photosynthetic compounds (Sage 1994). Our results suggest that a limited, albeit expanding, pool of plant N was diluted by more rapid plant growth, and that the capacity for enhanced C gain at elevated CO_2 did not completely compensate for the

Table 4. Nodule volume density and mass and N_2 fixation per unit of nodule mass for *A. smallii* grown for 13 months at each of three CO_2 concentrations. Values for each CO_2 treatment are means for three 380-liter soil containers each with three plants. Means within a row do not differ significantly ($P > 0.05$) if followed by the same letter.

Parameter	Growth CO_2 concentration (μmol mol^{-1})		
	385	690	980
Nodule volume density (nodules cm^{-3})	0.033 a	0.056 a	0.196 b
Nodule mass/volume (mg cm^{-3})	0.064 a	0.096 a	0.169 b
N_2 fixation/nodule mass (g N (g nodule)$^{-1}$)	0.444 a	0.862 b	0.664 ab

limited N resources in *A. smallii*. It is commonly observed that, following growth at elevated CO_2, tissue N concentrations decline in plants that do not symbiotically fix N_2 (Larigauderie et al. 1988, Norby et al. 1992). Insufficient data are available to establish trends in leaf N concentration or in the relationship of leaf N to photosynthesis in N_2-fixing woody species. Nitrogen per unit leaf mass declined at elevated CO_2 in actinorhizal and leguminous woody plants (Norby 1987, Arnone and Gordon 1990, Thomas et al. 1991), but N per unit leaf area in the woody legume *Gliricidia sepium* (Jacq.) Kunth ex Walp. increased or remained constant at elevated CO_2 (Thomas et al. 1991). In contrast, Vogel and Curtis (1995) reported an increase in leaf photosynthetic capacity of *Alnus glutinosa* L. Gaertn. (black alder) at elevated CO_2 concentration that was correlated with a late season increase in N per unit leaf area.

Down-regulation of photosynthesis was compensated by downward adjustments in stomatal conductance at elevated CO_2 (Figure 3), to maintain a nearly constant c_i/c_a ratio. There is limited evidence from gas exchange studies on single leaves that stomatal (or leaf) conductance at a given CO_2 concentration is decreased in some species following growth at elevated CO_2 concentrations (Imai and Murata 1978, DeLucia et al. 1985, Peet et al. 1986, Spencer and Bowes 1986, Woodward 1987, Berryman et al. 1994). It is not clear, however, whether changes in conductance parallel those in photosynthetic capacity.

Although potential productivity of *A. smallii* was reduced by greater leaf turnover at elevated CO_2 concentrations, plants grown at elevated CO_2 concentrations had a similar fraction of both aboveground and total biomass in leaves at harvest as plants grown at ambient CO_2 concentration. Because specific leaf area did not differ with CO_2 treatment, there was no effect of CO_2 on leaf area ratio (leaf area/whole-plant biomass) at harvest. Therefore, the potential growth response of *A. smallii* to CO_2 was apparently not limited by reduced distribution of plant biomass to leaf mass at elevated CO_2 concentrations, as sometimes occurs in other woody species (e.g., Norby et al. 1992, Polley et al. 1994).

Many of the effects of plants on ecosystems are correlated with plant size or biomass (Chapin 1993), and could thus be

influenced by atmospheric CO$_2$ concentration. In grasslands, for example, large trees or shrubs alter the microclimatic conditions experienced by smaller competitors and often increase soil nutrient concentrations under their canopies (Belsky et al. 1989) by transporting resources acquired in adjacent areas through extensive lateral roots. As a result, production of neighboring herbaceous vegetation may be altered, initiating changes in the frequency or intensity of fires and in the foraging activity of large herbivores.

Vitousek (1990) identified three mechanisms by which species invasions or additions may alter ecosystem function: (1) changes in disturbance frequencies or intensities; (2) changes in trophic structure; and (3) shifts in rates and patterns of resource acquisition, availability, and use. The latter effect of woody species invasion may be of greatest importance on grasslands, and is also directly sensitive to atmospheric CO$_2$ concentration.

Low-N availability of soil frequently limits production on grasslands (Schimel et al. 1991, Seastedt et al. 1991) and may also limit the response of net primary production to changes in CO$_2$ concentration. The enormous effect that elevated CO$_2$ had on N$_2$ fixation in *A. smallii* is therefore significant. Plants grown at 690 μmol mol^{-1} CO$_2$ fixed more N than was harvested in total from plants grown near the ambient CO$_2$ concentration. The amount of N fixed per plant at the highest CO$_2$ concentration exceeded total N accretion by plants grown at 385 μmol mol^{-1} CO$_2$ by a factor of 1.5. This positive effect of CO$_2$ on N$_2$ fixation by *A. smallii* during its initial year of growth is generally consistent with that reported for woody legumes (Thomas et al. 1991, Vogel and Curtis 1995), although most previous experiments with woody species have considered smaller plants and shorter growth periods. Nitrogen fixation promoted and sustained the growth response to elevated CO$_2$, and would be expected to provide an advantage for *A. smallii* in competition with non N$_2$-fixing species in N-poor soil. Ultimately, N addition to the soil following litterfall, root turnover and exudation, or mortality of the legume should enhance the productivity of a grassland ecosystem. Because the increase in N$_2$ fixation was measured under well-watered conditions, it should be regarded as near maximal. Nevertheless, stimulation of N$_2$ fixation may be among the more important ways by which increasing atmospheric CO$_2$ concentration modifies the impact of woody legumes on grasslands.

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